

Novel alcohol dehydrogenases

The invention relates to novel polypeptides which have the biological activity of an NAD- or NADP-dependent alcohol dehydrogenase. The invention furthermore relates to nucleic acids encoding said polypeptides, to nonhuman hosts or host cells and to reaction systems which may be used for preparing desired products. The polypeptides of the invention are preferably used in the preparation, starting from aldehydes or ketones, of primary and enantiomerically pure secondary alcohols which may serve as intermediates for medicaments. Alternatively, the polypeptides of the invention may also be employed in the reverse reaction, i.e. the oxidation of alcohols with the formation of aldehydes or ketones.

The description makes reference to a number of documents. The disclosure content of these documents is hereby incorporated by reference.

Enantiomerically pure alcohols are among the most important chiral building blocks of industrial special and fine chemistry. These products act, inter alia, as essential key intermediates in the preparation of medicaments. For a long time, the industrial route to these target molecules went preferably via purely chemical processes, for example by way of resolution of the racemate. This involves, starting from a ketone, firstly preparing the alcohol in its racemic form and then isolating the desired enantiomer in a resolution of the racemate with the aid of at least stoichiometric amounts of a chiral auxiliary substance. Disadvantages of these methods include not only the 50% maximum yield of the resolution of the racemate but must also be seen in the use of numerous ecologically problematic starting compounds for preparing the racemate. Further disadvantages are the additional step of recycling the undesired enantiomer as well as the need of chiral

auxiliary reagents (moreover, stoichiometric amounts thereof) for the resolution of the racemate. The concept of the resolution of the racemate is summarized in the equation (1a) in the overview in Figure 1. A first
5 substantial progress toward a more sustainable process was achieved using the biocatalytic resolution of the racemate, thereby dispensing with the necessity of employing stoichiometric amounts of chiral auxiliary reagents.
Regrettably, however, all other disadvantages listed above
10 remained relevant, despite such a biocatalytic route.

One possible way of avoiding the above-described disadvantages of the resolution of the racemate or of diastereoselective syntheses is the direct conversion of
15 ketones to the desired optically active alcohols in one step. Such "direct asymmetrical processes" may be carried out firstly by using metal-containing chemocatalysts, with the chemocatalysts employed being heavy metal-containing complexes which include a chiral ligand. Besides the use of
20 ecologically problematic heavy metals as a substantial catalyst component, the need for expensive and partly very sensitive ligands, for example phosphane ligands, is also disadvantageous.
25 Another alternative is the direct asymmetrical reduction using suitable biocatalysts for quantitative conversion of prochiral substrates to the desired enantiomerically pure product. Here too, the number of reaction steps is reduced to the theoretically possible minimum of only one step, the
30 biocatalytic conversion is carried out under ecologically excellent conditions (inter alia water as a solvent), and the process as such proceeds with high atom economy. The concept of a biocatalytic and sustainable process of this kind is set out in the equation (1b) of the overview in
35 Figure 1.

One disadvantage of the biocatalytic variant, however, is the lack of alcohol dehydrogenases available on an industrial scale as suitable biocatalysts for the target reaction and expression thereof. The object on which the 5 present invention is based was therefore to obtain novel, efficient and industrially usable alcohol dehydrogenases. This object is achieved by the embodiments characterized in the claims.

10 Thus, the invention relates to a polypeptide which has the biological activity of an NAD- or NADP-dependent alcohol dehydrogenase and which comprises or has one of the following sequences: the sequence of SEQ ID NO.: 1, the sequence of SEQ ID NO.: 2, the sequence of SEQ ID NO.: 3 or 15 a sequence which is at least 90% identical to the sequence of SEQ ID NO.: 3. Said sequence is preferably at least 95% identical to SEQ ID NO.: 3. More preferably, said sequence is at least 98% or 99% identical to SEQ ID NO.: 3. Also comprised is the sequence of SEQ ID NO.: 4. Furthermore 20 comprised is the sequence of SEQ ID NO.: 5 or a sequence which is at least 90% identical to the sequence of SEQ ID NO.: 5. Preferably, said sequence is at least 95% identical to SEQ ID NO.: 5. More preferably, said sequence is at least 98% or 99% identical to SEQ ID NO.: 5. Also comprised 25 is the sequence of SEQ ID NO.: 6 or a sequence which is at least 90% identical to the sequence of SEQ ID NO.: 6. Preferably, said sequence is at least 95% identical to SEQ ID NO.: 6. More preferably, said sequence is at least 98% or 99% identical to SEQ ID NO.: 6. Likewise comprised is 30 the sequence of SEQ ID NO.: 7 or a sequence which is at least 70% identical to the sequence of SEQ ID NO.: 7. Preferably, said sequence is at least 75% identical to SEQ ID NO.: 7. More preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 7. Even more 35 preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 7. Also comprised is the sequence of SEQ ID NO.: 8 or a sequence

which is at least 70% identical to the sequence of SEQ ID NO.: 8. Preferably, said sequence is at least 75% identical to SEQ ID NO.: 8. More preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 8. Even 5 more preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 8.

Additionally comprised is the sequence of SEQ ID NO.: 9 or a sequence which is at least 70% identical to the sequence 10 of SEQ ID NO.: 9. Preferably, said sequence is at least 75% identical to SEQ ID NO.: 9. More preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 9. Even more preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 9. 15 Likewise comprised is the sequence of SEQ ID NO.: 10 or a sequence which is at least 70% identical to the sequence of SEQ ID NO.: 10. Preferably, said sequence is at least 75% identical to SEQ ID NO.: 10. More preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 10. Even more preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 10. Also comprised is the sequence of SEQ ID NO.: 11 20 or a sequence which is at least 70% identical to the sequence of SEQ ID NO.: 11. Preferably, said sequence is at least 75% identical to sequence SEQ ID NO.: 11. More preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 11. Even more preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 11. Furthermore comprised is 25 the sequence of SEQ ID NO.: 12 or a sequence which is at least 60% identical to the sequence of SEQ ID NO.: 12. Preferably, said sequence is at least 65% identical to SEQ ID NO.: 12. More preferably, said sequence is at least 70% identical to the sequence of SEQ ID NO.: 12. Even more 30 preferably, said sequence is at least 75% or 80% identical to SEQ ID NO.: 12. Particularly preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the 35

sequence of SEQ ID NO.: 12. Likewise comprised is the sequence of SEQ ID NO.: 13 or a sequence which is at least 60% identical to the sequence of SEQ ID NO.: 13. Preferably, said sequence is at least 65% identical to SEQ 5 ID NO.: 13. More preferably, said sequence is at least 70% identical to the sequence of SEQ ID NO.: 13. Even more preferably, said sequence is at least 75% or 80% identical to SEQ ID NO.: 13. Particularly preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the 10 sequence of SEQ ID NO.: 13. Also comprised is the sequence of SEQ ID NO.: 14 or a sequence which is at least 75% identical to the sequence of SEQ ID NO.: 14. Preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 14. More preferably, said sequence is at least 85% identical to the sequence of SEQ ID NO.: 14. Even more 15 preferably, said sequence is at least 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 14. Additionally comprised is the sequence of SEQ ID NO.: 15 or a sequence which is at least 95% identical to the sequence of SEQ ID NO.: 15. Preferably, said sequence is at least 98% or 99% 20 identical to the sequence of SEQ ID NO.: 15. Also comprised is the sequence of SEQ ID NO.: 16 or a sequence which is at least 95% identical to the sequence of SEQ ID NO.: 16. Preferably, said sequence is at least 98% or 99% identical to the sequence of SEQ ID NO.: 16. Furthermore comprised is 25 the sequence of SEQ ID NO.: 17 or a sequence which is at least 75% identical to the sequence of SEQ ID NO.: 17. Preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 17. More preferably, said sequence 30 is at least 85% identical to the sequence of SEQ ID NO.: 17. Even more preferably, said sequence is at least 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 17. Likewise comprised is the sequence of SEQ ID NO.: 18 or a sequence which is at least 70% identical to the sequence 35 of SEQ ID NO.: 18. Preferably, said sequence is at least 75% identical to SEQ ID NO.: 18. More preferably, said sequence is at least 80% identical to the sequence of SEQ

ID NO.: 18. Even more preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 18. Also comprised is the sequence of SEQ ID NO.: 19 or a sequence which is at least 70% identical to the sequence of SEQ ID NO.: 19. Preferably, said sequence is at least 75% identical to SEQ ID NO.: 19. More preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 19. Even more preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 19. Additionally comprised is the sequence of SEQ ID NO.: 20 or a sequence which is at least 60% identical to the sequence of SEQ ID NO.: 20. Preferably, said sequence is at least 65% identical to SEQ ID NO.: 20. More preferably, said sequence is at least 70% identical to the sequence of SEQ ID NO.: 20. Even more preferably, said sequence is at least 75% or 80% identical to SEQ ID NO.: 20. Particularly preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 20. Also comprised is the sequence of SEQ ID NO.: 21 or a sequence which is at least 90% identical to the sequence of SEQ ID NO.: 21. Preferably, said sequence is at least 95% identical to the sequence of SEQ ID NO.: 21. More preferably, said sequence is at least 98% or 99% identical to the sequence of SEQ ID NO.: 21. Furthermore comprised is the sequence of SEQ ID NO.: 22 or a sequence which is at least 70% identical to the sequence of SEQ ID NO.: 22. Preferably, said sequence is at least 75% identical to SEQ ID NO.: 22. More preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 22. Particularly preferably said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 22. Likewise comprised is the sequence of SEQ ID NO.: 23 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 23. Preferably, said sequence is at least 60% identical to SEQ ID NO.: 23. More preferably, said sequence is at least 65% identical to the sequence of SEQ ID NO.: 23. Even more preferably, said sequence is at least 70% or

75% identical to SEQ ID NO.: 23. Particularly preferably, said sequence is at least 80%, 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 23. Also comprised is the sequence of SEQ ID NO.: 24 or a sequence which is at 5 least 65% identical to the sequence of SEQ ID NO.: 24. Preferably, said sequence is at least 70% identical to SEQ ID NO.: 24. More preferably, said sequence is at least 75% identical to the sequence of SEQ ID NO.: 24. Even more preferably, said sequence is at least 80% or 85% identical 10 to SEQ ID NO.: 24. Particularly preferably, said sequence is at least 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 24. Furthermore comprised is the sequence of SEQ ID NO.: 25 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 25. Preferably, 15 said sequence is at least 60% identical to SEQ ID NO.: 25. More preferably, said sequence is at least 65% identical to the sequence of SEQ ID NO.: 25. Even more preferably, said sequence is at least 70% or 75% identical to SEQ ID NO.: 25. Particularly preferably, said sequence is at least 80%, 20 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 25. Also comprised is the sequence of SEQ ID NO.: 26 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 26. Preferably, said sequence is at least 60% identical to SEQ ID NO.: 26. More preferably, 25 said sequence is at least 65% identical to the sequence of SEQ ID NO.: 26. Even more preferably, said sequence is at least 70% or 75% identical to SEQ ID NO.: 26. Particularly preferably, said sequence is at least 80%, 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 26. 30 Likewise comprised is the sequence of SEQ ID NO.: 27 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 27. Preferably, said sequence is at least 60% identical to SEQ ID NO.: 27. More preferably, said sequence is at least 65% identical to the sequence of SEQ ID NO.: 27. Even more preferably, 35 said sequence is at least 70% or 75% identical to SEQ ID NO.: 27. Particularly preferably, said sequence is at least 80%, 85%, 90%, 95%, 98% or 99%

identical to the sequence of SEQ ID NO.: 27. Furthermore comprised is the sequence of SEQ ID NO.: 28 or a sequence which is at least 75% identical to the sequence of SEQ ID NO.: 28. Preferably, said sequence is at least 80%

5 identical to SEQ ID NO.: 28. More preferably, said sequence is at least 85% identical to the sequence of SEQ ID NO.: 28. Even more preferably, said sequence is at least 90%, 95%, 98% or 99% identical to SEQ ID NO.: 28. Likewise comprised is the sequence of SEQ ID NO.: 29 or a sequence

10 which is at least 70% identical to the sequence of SEQ ID NO.: 29. Preferably, said sequence is at least 75% identical to SEQ ID NO.: 29. More preferably, said sequence is at least 80% identical to the sequence of SEQ ID NO.: 29. Even more preferably, said sequence is at least 85% or

15 90% identical to SEQ ID NO.: 29. Particularly preferably, said sequence is at least 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 29. Also comprised is the sequence of SEQ ID NO.: 30 or a sequence which is at least 60% identical to the sequence of SEQ ID NO.: 30. Preferably,

20 said sequence is at least 65% identical to SEQ ID NO.: 30. More preferably, said sequence is at least 70% identical to the sequence of SEQ ID NO.: 30. Even more preferably, said sequence is at least 75% or 80% identical to SEQ ID NO.: 30. Particularly preferably, said sequence is at least 85%,

25 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 30. Furthermore comprised is the sequence of SEQ ID NO.: 31 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 31. Preferably, said sequence is at least 60% identical to SEQ ID NO.: 31. More

30 preferably, said sequence is at least 65% or 70% identical to the sequence of SEQ ID NO.: 31. Even more preferably, said sequence is at least 75% or 80% identical to SEQ ID NO.: 31. Particularly preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ

35 ID NO.: 31. Likewise comprised is the sequence of SEQ ID NO.: 32 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 32. Preferably, said sequence

is at least 60% identical to SEQ ID NO.: 32. More preferably, said sequence is at least 65% or 70% identical to the sequence of SEQ ID NO.: 32. Even more preferably, said sequence is at least 75% or 80% identical to SEQ ID NO.: 32. Particularly preferably, said sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO.: 32. Furthermore comprised is the sequence of SEQ ID NO.: 33 or the sequence of SEQ ID NO.: 34. The amino acid sequences mentioned and characterized by SEQ IDs are 10 preferably encoded by the DNA sequences referred to as SEQ ID numbers 35 to 68. Preference is further given to polypeptides which correspond to the naturally occurring enzymes over their full length. In another preferred embodiment, the polypeptides of the invention additionally comprise at least one heterologous amino acid section which 15 characterizes said polypeptides as fusion proteins.

Possible examples of heterologous components of the fusion protein of the invention are Tags (e.g. His-Tag or Flag-Tag) which may be used for purification of the fusion 20 proteins of the invention. In other embodiments, the heterologous components may have their own enzymic activity. In such a case, the two enzymic components are preferably linked by a linker such as a flexible glycine or glycine-serine linker of 6-10 amino acids in length, in 25 order to ensure the functionality of said components. The term "heterologous", as used herein, may mean, firstly, that the components of the fusion protein do not naturally occur covalently linked together and, secondly, that the components originate from different species. Fusion 30 proteins are usually prepared using recombinant DNA technology (see Sambrook et al., loc. cit.).

According to the invention, the term "polypeptide which has the biological activity of an NAD- or NADP-dependent 35 alcohol dehydrogenase" refers to a group of enzymes which catalyze the conversion of alcohols to aldehydes or ketones or the corresponding reverse reaction, i.e. the conversion

of aldehydes to primary alcohols or ketones to secondary alcohols. The first-mentioned reaction corresponds in this connection to an oxidative process, with the secondly mentioned type of reaction being a reductive process. The 5 EC number of alcohol dehydrogenases (ADHs) is EC 1.1.1.1. The scope of protection of the invention comprises, in addition to the naturally occurring enzymes isolated in the course of the present invention, also those polypeptides which have the aforementioned identity values at the amino 10 acid level compared to the polypeptides isolated from natural sources and which may likewise originate from natural sources. On the other hand, they may be modified by recombinant DNA technology in such a way that the enzymic activity is retained or essentially retained, as will be 15 anticipated by the skilled worker (cf., for example, Sambrook et al, "Molecular Cloning, A Laboratory Handbook", 2nd edition 1989, CSH Press, Cold Spring Harbor, Ausubel et al. "Current Protocols in Molecular Biology", John Wiley & Sons, NY 2001). Thus, it is possible for amino acids which 20 are not located at the active site and whose replacement with an amino acid "of the same kind" is not expected at first sight to result in a substantially altered three-dimensional structure to be replaced with an amino acid "of the same kind". For example, particular amino acids with 25 nonpolar side chains (amino acids of the same kind), may be expected to be able to be substituted, for example valine for alanine, without this having a (substantial) influence on the biological function of the enzyme, on the enzymic activity in accordance with the invention. On the basis of 30 his specialist knowledge, the skilled worker may draw corresponding conclusions also for the substitution of other types of amino acids (for example the replacement of basic amino acids with other basic amino acids or of amino acids with uncharged polar side chains with other amino 35 acids from this group).

The percentage of identity to the amino acid sequences of the polypeptides isolated from natural sources, which are described in this description by SEQ ID numbers, may be readily determined by the skilled worker using processes
5 known in the prior art. A suitable program which may be used according to the invention is BLASTP (Altschul et al.. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25(17):3389-3402.).

10

The invention also relates to a nucleic acid molecule which encodes the polypeptide of the invention.

15 The nucleic acid molecule of the invention may be a DNA molecule or an RNA molecule. Preference is given to the nucleic acid molecule being a cDNA molecule or an mRNA molecule. According to the invention, said DNA molecule may furthermore be a genomic DNA molecule. The invention also comprises embodiments in which said DNA molecule is a PNA molecule or another derivative of a DNA molecule. According to the invention, preference is given to DNA sequences comprising the DNA sequences according to SEQ ID numbers 35
20 to 68.

25 In order to achieve the object on which the invention is based, the following approach was pursued. Firstly, based on an extensive proprietary strain collection, prioritized strains were grown on plates and, after viability and purity controls had been carried out, also in liquid
30 culture. The genomic DNA of these organisms was isolated from the harvested cell pellets. Based on the genomic DNA prepared, selected isolates were genetically screened for alcohol dehydrogenase genes via PCR typing by means of primers of the invention. In this context, even the amino
35 acid sequence similarity due to homology of already known alcohol dehydrogenases did not readily allow oligonucleotide primers to be derived with the aid of which

previously unidentified alcohol dehydrogenase genes may readily be amplified successfully. Initially, this approach was based on the hypothesis of particular sequence motifs conserved in the previously known alcohol dehydrogenase genes also being present in the desired novel alcohol dehydrogenase genes. However, the sequence motifs conserved in the previously known alcohol dehydrogenase genes are unsuitable for deriving degenerated primers by processes known to the skilled worker (Kwok et al.. 1995. Design and 5 use of mismatched and degenerate primers. In: PCR Primer , A laboratory Manual, Dieffenbach CW & Dveksler GS (Editors), Cold Spring Habor Laboratory Press, pp143-155; Compton T. 1990. Degenerate Primers for DNA Amplification. In: PCR Protocols, A Guide to Methods and Applications. 10 Innis et al. (Editors) Academic Press, San Diego, pp39-34). The NAD- or NADP-dependent alcohol dehydrogenases are classified as long-chain, medium-chain and short-chain ADHs. They are divided into these three groups especially based on their metal dependence and the size of subunits. 15 The short-chain ADHs do not require any metal ions and their subunits consist of approximately 250 amino acids. In contrast, the medium-chain and long-chain ADHs are dependent on metal ions. The medium-chain ones whose typical subunits consist of approx. 350 amino acids require zinc ions. The long-chain ADHs whose subunits are composed 20 of approx. 385 amino acids require iron ions (Hummel, W. 1997. New alcohol dehydrogenases for the synthesis of chiral compounds. 58:145-84). The sequence heterogeneity, not only within all of the previously known NAD- or NADP- 25 dependent ADHs but also within the three ADH groups briefly described above, is extremely high. Therefore, it was not that easy to construct primers with the aid of which it is possible firstly to amplify specifically ADH sequences and secondly also to capture a diversity of novel ADHs 30 necessary in order to achieve the object. Thus, despite the sequence homologies expected on the basis of the used, no long-chain ADHs whatsoever were isolated in the bacteria 35

studied. In this connection, it was intended to test the quality of the constructed primers first with genomic DNA of model organisms whose alcohol dehydrogenase genes are known or with DNA pools consisting of DNA from various
5 microorganisms. This involved cloning, sequencing and subsequently analyzing PCR products. After this establishing phase, selected isolates were subjected to PCR typing on the basis of the prepared genomic DNA of the microorganisms to be screened, as described above. The
10 results obtained from the experiments (regarding sequence identity and specific activity) were incorporated into prioritizing the potential Hit organisms whose novel alcohol dehydrogenase genes are being isolated. Despite the unexpected, disappointing and demotivating results in the
15 course of the attempted isolation of nucleic acids supposed to encode long-chain ADHs, a number of nucleic acids encoding short-chain enzymes and medium-chain enzyme chains were isolated according to the invention. Some of these enzymes and enzyme chains had surprisingly low sequence
20 identities (<50%) to the known enzymes of this class.

The invention furthermore relates to a nucleic acid molecule which is complementary to the nucleic acid molecule of the invention.

25 According to the invention, the term "complementary" means a complementarity which extends across the entire region of the nucleic acid molecule of the invention without gaps. In other words, preference is given according to the invention
30 to said complementarity extending 100% across the entire region of the sequence of the invention, i.e. from the 5' end shown to the 3' end shown. In further preferred embodiments, said complementarity extends across a region of at least 19, preferably at least 21, contiguous
35 nucleotides which preferably do not code for the active site of enzymic activity.

In addition, the invention relates to a vector which comprises the nucleic acid molecule of the invention.

The vectors of the invention preferably contain the nucleic acids of the invention operatively linked to an expression control sequence so as for said nucleic acids to be able to be transcribed and, where appropriate, translated in a suitable host cell. Expression control sequences usually comprise a promoter and, where appropriate, further regulatory sequences such as operators or enhancers.

Furthermore, translation initiation sequences may also be present. Suitable expression control sequences for prokaryotic or eukaryotic host cells are known to the skilled worker (see, for example, Sambrook et al., loc. cit.). The recombinant vector of the invention may furthermore also contain usual elements such as an origin of replication and a selection marker gene. Examples of suitable recombinant vectors are plasmids, cosmids, phages or viruses (see, for example, Sambrook et al., supra).

Starting materials for preparing the recombinant vectors of the invention are commercially available (for example from Stratagene, InVitroGen or Promega).

Any plasmids or vectors available to the skilled worker for this purpose are suitable in principle. Plasmids and vectors of this kind may be found, for example, in Studier and coworkers (Studier, W. F.; Rosenberg A. H.; Dunn J. J.; Dubendorff J. W.; (1990), Use of the T7 RNA polymerase to direct expression of cloned genes, Methods Enzymol. 185, 61-89) or in the brochures from Novagen, Promega, New England Biolabs, Clontech or Gibco BRL. Further preferred plasmids and vectors may be found in: Glover, D. M. (1985), DNA cloning: a practical approach, Vol. I-III, IRL Press Ltd., Oxford; Rodriguez, R.L. and Denhardt, D. T (eds) (1988), Vectors: a survey of molecular cloning vectors and their uses, 179-204, Butterworth, Stoneham; Goeddel, D. V. (1990), Systems for heterologous gene expression, Methods

Enzymol. 185, 3-7; Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989), Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York. Plasmids which may be used for cloning the gene 5 construct having the nucleic acid of the invention into the host organism in a very preferred manner are derivatives of: pUC18 and pUC19 (Roche Biochemicals), pKK-177-3H (Roche Biochemicals), pBTac2 (Roche Biochemicals), pKK223-3 (Amersham Pharmacia Biotech), pKK-233-3 (Stratagene) or pET 10 (Novagen). Other preferred plasmids are pBR322 (DSM3879), pACYC184 (DSM4439) and pSC101 (DSM6202), which may be obtained from the DSMZ-Deutsche Sammlung von 15 Mikroorganismen und Zellkulturen GmbH, Brunswick, Germany. Examples of preferred promoters are T7, lac, tac, trp, rha and ara.

The invention also relates to a nonhuman host which comprises the polypeptide of the invention or the nucleic acid molecule of the invention or the vector of the 20 invention.

The nonhuman host may be a cell or a multi- to polycellular organism. Suitable polycellular organisms include model systems familiar in molecular biology, such as Drosophila 25 melanogaster, zebra fish or C. elegans.

In a preferred embodiment, the host is a cell.

In this preferred embodiment, the host of the invention is 30 a recombinant cell which has been transformed or transfected with a nucleic acid of the invention or a vector of the invention (according to the present invention, the terms "transformation" and "transfection" are used synonymously). Transformation or transfection may 35 be carried out by known methods, for example calcium phosphate coprecipitation, lipofection, electroporation, particle bombardment or viral infection. The cell of the

invention may contain the recombinant nucleic acid in an extrachromosomal or a chromosomally integrated form. In other words, the transfection/transformation may be stable or transient.

5

The recombinant cell preferably is of prokaryotic origin. Suitable host cells include cells of unicellular microorganisms, such as bacteria cells. A particularly suitable bacterial host system is *E. coli*. The cytoplasm of *E. coli* contains the cofactors required for the enzymic activity of the polypeptide of the invention. These are, in particular, NADH, NADPH, NAD⁺ and NADP⁺. Very particular preference is given to: *E. coli* XL1 Blue, W3110, DSM14459 (PCT/US00/08159), NM 522, JM101, JM109, JM105, RR1, DH5, TOP 10- or HB101. It is also possible to use for expression of the nucleic acids of the invention bacteria of the genera/species *Lactobacillus*, *Bacillus*, *Rhodococcus*, *Campylobacter*, *Caulobacter*, *Mycobacterium*, *Streptomyces*, *Neisseria*, *Ralstonia*, *Pseudomonas*, and *Agrobacterium*.

Appropriate strains are available in the prior art and may, at least partially, be obtained via the international deposition sites such as ATCC or DMSZ. Transfection protocols and transformation protocols are known to the skilled worker. (Chan and Cohen. 1979. High Frequency Transformation of *Bacillus subtilis* Protoplasts by Plasmid DNA. *Mol Gen Genet.* 168(1):111-5; Kieser et al.. 2000. Practical *Streptomyces* Genetics. The John Innes Foundation Norwich.; Sambrook et al.. 1989. Molecular Cloning. A Laboratory Manual. In: second ed.. Cold Spring Harbor Laboratory Press. Cold Spring Harbor. NY.; Irani and Rowe. 1997. Enhancement of transformation in *Pseudomonas aeruginosa* PAO1 by Mg²⁺ and heat. *Biotechniques* 22: 54-56). Additional examples of host organisms which may be used are also yeasts such as *Hansenula polymorpha*, *Pichia* sp., *Saccharomyces cerevisiae*.

As an alternative to this, the cell may be of eukaryotic origin. Suitable eukaryotic cells include CHO cells, HeLa cells and others. Many of these cells are obtainable via deposition sites such as ATCC or DMSZ.

- 5 In a further preferred embodiment, the host is a transgenic nonhuman animal.

Transgenic nonhuman animals may be produced by processes known in the prior art.

- 10 The transgenic nonhuman animal of the invention may preferably have various genetic constitutions. It may (i) constitutively or inducibly overexpress the gene of a nucleic acid of the invention, (ii) contain the endogenous gene of a nucleic acid of the invention in an inactive form, (iii) contain the endogenous gene of a nucleic acid of the invention completely or partially replaced with a mutated gene of a nucleic acid of the invention, (iv) have conditional and tissue-specific overexpression or
15 underexpression of the gene of a nucleic acid of the invention or (v) have a conditional and tissue-specific knock-out of the gene of a nucleic acid of the invention.
20

- 25 Preferably, the transgenic animal additionally contains an exogenous gene of a nucleic acid of the invention under the control of a promoter allowing overexpression.

- 30 Alternatively, the endogenous gene of a nucleic acid of the invention may be overexpressed by activating or/and replacing the intrinsic promoter. Preferably, the endogenous promoter of the gene of a nucleic acid of the invention has a genetic modification which results in increased expression of the gene. Said genetic modification of the endogenous promoter here comprises both a mutation
35 of individual bases and deletion and insertion mutations.

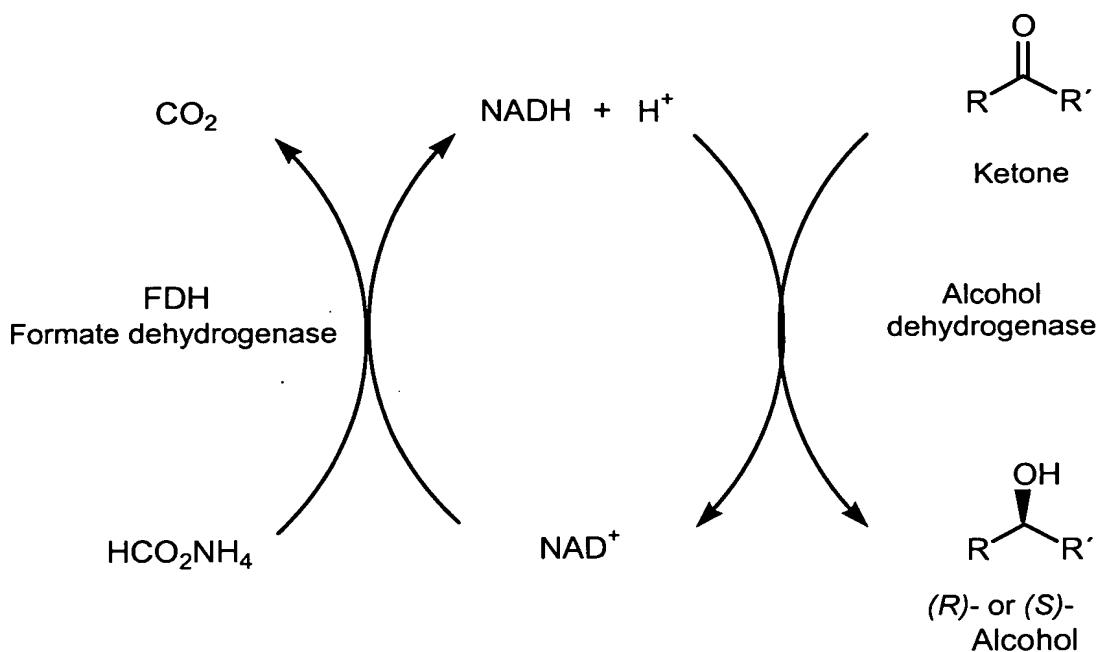
In a particularly preferred embodiment of the host of the invention, the latter is a transgenic rodent, preferably a transgenic mouse, a transgenic rabbit, a transgenic rat, or 5 is a transgenic sheep, a transgenic cow, a transgenic goat or a transgenic pig.

Mice have numerous advantages over other animals. They can be kept easily and their physiology is regarded as a model system for that of humans. The production of such gene-manipulated animals is sufficiently known to the skilled 10 worker and carried out using common processes (see, for example, Hogan, B., Beddington, R., Costantini, F. and Lacy, E. (1994), Manipulating the Mouse-Embryo; A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, 15 Cold Spring Harbor, NY; WO91/08216).

Alternatively or additionally, it is also possible to employ cell culture systems, in particular human cell culture systems, for the applications described for the 20 nonhuman transgenic animal of the invention.

Examples of cofactors of alcohol dehydrogenases, which are used - as already mentioned, depending on the particular alcohol dehydrogenase - are NADH and NADPH and their 25 oxidized forms, NAD⁺ and NADP⁺, respectively.

The cofactors may be regenerated, in principle, either in an enzyme-coupled manner using a second enzyme, for example a formate dehydrogenase or glucose dehydrogenase, or in a 30 substrate-coupled manner using any of the alcohols accepted as substrate by the alcohol dehydrogenase employed, for example - iso-propanol - if accepted as substrate. The diagram below indicates by way of example the concept of the alcohol dehydrogenase-catalyzed 35 reduction of a ketone with enzyme-coupled cofactor regeneration using a formate dehydrogenase.



Alcohol dehydrogenases are used, for example, for preparing enantiomerically enriched, preferably enantiomerically

5 pure, secondary alcohols, starting from prochiral ketones. In this connection, both (R) - and (S) -specific alcohol dehydrogenases are known which accordingly result in the formation of the particular enantiomeric (R) and, respectively, (S) forms of alcohols.

10

Correspondingly, preference is also given according to the invention to a host which has a further dehydrogenase suitable for cofactor regeneration or a nucleic acid molecule encoding said dehydrogenase.

15

In this connection, the host may contain naturally said further dehydrogenase or may have been transfected with a recombinant nucleic acid which encodes said dehydrogenase and with which it is possible to express said dehydrogenase 20 in said host. This embodiment also requires the host having the cofactors necessary for the function of said further dehydrogenase or said cofactors being delivered to said host in a suitable manner.

Particular preference is given in this connection to a host in which the dehydrogenase suitable for cofactor regeneration is a formate dehydrogenase or a glucose dehydrogenase. Particular preference is given to a *Candida boidinii* formate dehydrogenase. Particular preference is also given to the cofactor-regenerating dehydrogenase being a *Bacillus subtilis* glucose dehydrogenase. Genetically modified mutants of said cofactor-regenerating dehydrogenases, which retain said enzymic function, are likewise preferred according to the invention.

In a further embodiment, the invention relates to a reaction system which comprises an organic compound which is a substrate of a dehydrogenase, furthermore the polypeptide of the invention, the vector of the invention or the host of the invention and, where appropriate, a cofactor for the polypeptide of the invention. (The addition of cofactor is required in those cases in which the cofactor is not already present in the system, see also hereinbelow). In one case, the reaction system of the invention may be a bacterial cell which corresponds to the host of the invention and which has the polypeptide of the invention and also the necessary cofactors in the cytoplasm. In the case of the cofactor(s) already being present naturally in the system/host, said cofactors need no longer be delivered separately. Suitably, the host is one which has a further dehydrogenase suitable for cofactor regeneration or a nucleic acid molecule encoding said dehydrogenase and also the cofactors required therefor. If a substrate for a desired product is supplied to said reaction system or if said substrate is metabolized in the reaction system itself, then the desired product may readily be isolated from the reaction system, if said reaction system is maintained under suitable conditions.

Suitable conditions include carrying out the reaction at temperatures of from 10 to 80°C, preferably from 20 to 60°C, and very preferably from 20 to 40°C. Preference is

- also given to the substrate concentration being from 100 to 2000 mM, preferably from 200 to 800 mM. In a preferred form, the desired reaction is carried out so as to achieve conversions of >80%, in particular >90%, within a reaction time of <20 hours, in particular a reaction time of <10 hours and very preferably a reaction time of <5 hours.
- In another embodiment, the reaction system may be an in vitro system for converting a suitable substrate to obtain the desired product. For example, the polypeptide of the invention may be contacted with the cofactors mentioned and the substrate and, where appropriate (i.e. if necessary), with a further dehydrogenase suitable for cofactor regeneration (and, where appropriate, cofactors required therefor, in particular NADH and/or NADPH their oxidized forms) under suitable conditions, as set out above, for example, and over a sufficient period of time, so that the desired product may be generated. In this in vitro variant with utilization of isolated enzymes (in purified form or as crude extract) and addition of cofactors, these cofactor additions should, in accordance with an economical process control, be <0.01 equivalents (based on the amount of substrate employed), preferably <0.001 equivalents and very preferably <0.0005 equivalents.
- The "reaction system" may moreover also be a transgenic nonhuman animal to which a suitable substrate and, where appropriate, cofactors or/and said further dehydrogenase is fed or administered and which is capable of converting said substrate in suitable tissues. In another embodiment, the reaction system may also be a cellular membrane system in which the enzyme, the enzymes and, where appropriate, the cofactors are anchored.
- Further preference is given according to the invention to a reaction system in which the organic compound which is a substrate of a dehydrogenase is a carbonyl compound.

Particular preference is given to a reaction system in which the carbonyl compound is an aldehyde or a ketone.

This embodiment of the invention permits the preparation, 5 particularly preferred according to the invention, of technical grade alcohols which may be used, for example, as intermediates for the preparation of active compounds usable in medicaments.

10 Particular preference is given according to the invention to the ketone being an asymmetrically substituted ketone.

This embodiment of the invention is particularly preferred because the products generated in a corresponding reduction 15 have a center of chirality and may be obtained with high enantioselectivity. In general, the desired chiral secondary alcohols are obtained in an enantiomerically pure form with an enantiomeric excess of >99%.

20 In another preferred embodiment of the reaction system of the invention, the organic compound which is a substrate of a dehydrogenase is an alcohol. This variant is preferably suitable for preparing commercially important carbonyl compounds, for example ketones relevant in the field of 25 aroma chemicals. In addition, oxidation may also be utilized for the formation of enantiomerically pure, secondary alcohols by starting from a racemic alcohol as substrate and converting the undesired enantiomer into the ketone compound by enantioselective oxidation. The 30 remaining, desired enantiomer may then be isolated accordingly.

The alcohol is preferably a primary alcohol or a chiral secondary alcohol. In the first case, the product generated is an aldehyde, whereas in the second case the 35 corresponding ketones are formed.

Preference is also given according to the invention to the cofactor in the reaction system of the invention being NADH, NADPH, NAD⁺ or NADP⁺.

- 5 The invention also relates to a process for preparing the polypeptide of the invention or a polypeptide encoded by the nucleic acid molecule of the invention, which process comprises growing the host of the invention and isolating said polypeptide.

10

The polypeptide may be purified, for example, by conventional processes, for example by disrupting appropriate cells, for example by means of a "French press", by ion exchange, size selection or affinity chromatography etc. (Coligan et al.. Current Protocols in Protein Science, John Wiley & Sons, Inc.). As an alternative to this, the polypeptide of the invention, when linked to a leader peptide, may be exported out of the cells and purified from the culture supernatant. This embodiment requires the polypeptide of the invention, which does not naturally contain a leader peptide, to be genetically modified. This embodiment has the advantage of a simpler purification of the polypeptide of the invention from the culture supernatant. The best procedures and suitable leader peptides may be readily determined by the skilled worker.

15

20

25

30

35

In a further preferred embodiment of the process of the invention, the polypeptide is isolated from a body fluid or tissue sample of the nonhuman transgenic animal. In this embodiment too, the polypeptide of the invention preferably contains a leader peptide.

In a further preferred embodiment of the process of the invention and, in particular, if the nonhuman transgenic animal is a mammal, for example a cow, a goat or a sheep, the body fluid is milk or serum.

In another embodiment, the invention relates to a process for preparing an organic compound which is a product of a dehydrogenase, which process comprises reacting an organic 5 compound which is a substrate of a dehydrogenase with the polypeptide of the invention, the host of the invention or by means of the reaction system of the invention.

The various embodiments of the invention which are to be 10 used in the process of the invention differ in principle in that further components such as cofactors etc. (cf. supra) have to be added to the polypeptide, if the latter is used in a cell-free in vitro system. When using the reaction system of the invention, the necessary components, with the 15 possible exception of the substrate, are preferably and advantageously already present in the system, and a separate addition is thus not needed here.

Preference is given according to the invention to a process 20 which further comprises the step of isolating the product of the reaction. Suitable processes for isolation/purification have been set forth above.

In a particularly preferred embodiment of the process of 25 the invention, the latter further comprises processing the product to give a medicament. A number of descriptions of utilizing enantiomerically pure alcohols as intermediates for preparing pharmaceutical active compounds are given in the literature. An overview in this respect is contained, 30 inter alia, in: A. Kleemann, J. Engels, B. Kutscher, D. Reichert, Pharmaceutical Substances: Syntheses, Patents, Applications, 4th edition, Thieme-Verlag, Stuttgart, 2001.

In another, particularly preferred embodiment of the 35 process of the invention, the latter further comprises the step of processing the product to give a secondary product. In this connection, derivatization may take place both by

way of modification of the alcohol group, for example by esterification and subsequent secondary reactions, and by way of modifications of the particular substituents.

- 5 Particular preference is given here to the process of the invention further comprising the step of formulating the secondary product with a pharmaceutically compatible carrier or excipient or diluent in the preparation of a medicament.

10 Examples of suitable pharmaceutically compatible carriers and/or diluents are known to the skilled worker and comprise, for example, phosphate-buffered saline solutions, water, emulsions such as, for example, oil/water emulsions, 15 various types of wetting agents or detergents, sterile solutions, etc. Medicaments comprising such carriers may be formulated by means of known conventional methods. Said medicaments may be administered in a suitable dose to an individual. The administration may be carried out orally or 20 parenterally, for example intravenously, intraperitoneally, subcutaneously, intramuscularly, locally, intranasally, intrabronchially, orally or intradermally, or via a catheter at a site in an artery. Preparations for parenteral administration comprise sterile aqueous or 25 nonaqueous solutions, suspensions and emulsions. Examples of nonaqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as, for example, olive oil, and organic ester compounds such as, for example, ethyl oleate, which are suitable for injections. Aqueous carriers 30 comprise water, alcohol/water-based solutions, emulsions, suspensions, salt solutions and buffered media. Parenteral carriers comprise sodium chloride solutions, Ringer dextrose, dextrose and sodium chloride, Ringer lactate and bound oils. Examples of intravenous carriers include 35 liquid, nutrient and electrolyte supplements (such as, for example, those based on Ringer dextrose). The medicament may moreover comprise preservatives and other additives such

as, for example, antimicrobial compounds, antioxidants, complexing agents and inert gases. Depending on the intended specific usage, other active compounds such as, for example, interleukins, growth factors, differentiation factors, interferons, chemotactic proteins or an unspecific immunomodulating agent, may also be included.

The type of dosage is determined by the attending physician according to the clinical factors. The skilled worker knows that the type of dosage depends on various factors such as, for example, body size or weight, body surface area, age, sex or general health of the patient, or else on the agent to be specially administered, duration and type of administration, and on other medicaments which may be administered in parallel. A typical dose may be, for example, in a range between 0.001 and 1000 µg, with doses being conceivable below and above this exemplary range, especially when taking into account the abovementioned factors. If the composition of the invention is administered regularly, the unit dose per day should generally be in a range between 1 µg and 10 mg. The active compounds in these preparations are usually present at a concentration of more than 10 µg/ml of a physiological buffer. However, they may also be present in solid form at a concentration of from 0.1 to 99.5% by weight of the total mixture. In general, it has proven advantageous to administer the active compound(s) in total amounts of from about 0.001 to 100 mg/ kg, preferably in total amounts of from about 0.01 to 10 mg/kg, of body weight per 24 hours, where appropriate as continuous infusion or in the form of a plurality of individual doses, in order to achieve the desired result. If the composition is administered intravenously, the unit dose per kilogram of body weight per day should be in a range between 1 µg and 10 mg. The medicament may be administered topically, locally or systemically.

Finally, particular preference is given according to the invention to a process in which the product is an enantiomerically pure alcohol.

- 5 The invention also relates to a ligand which specifically binds the polypeptide of the invention, which ligand is neither a substrate of said polypeptide, nor a cofactor thereof, nor a product converted thereby.
- 10 The term "specifically binds" means according to the invention that the ligand does not or essentially does not cross react with other polypeptides, including those having a similar primary sequence or a similar three-dimensional structure. Cross reactivity may be determined by processes known in the prior art (cf. Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988). To this end, it is possible to use, for example, competitive assays, for example turbidimetric tests, in which the ligand is incubated together with the labeled polypeptide of the invention and a polypeptide competing therewith, it being possible for the latter to be used at different concentrations.
- 15
- 20

- 25 In a preferred embodiment, the ligand of the invention is an antibody or a fragment or derivative thereof, an aptamer, or a low-molecular weight substance.

- 30 Antibody fragments comprise Fv, Fab and F(ab')₂ fragments. The derivatives include scFvs (Harlow and Lane, loc. cit.). Antibodies may be of polyclonal or monoclonal origin.

- In a particularly preferred embodiment of the receptor of the invention, said receptor is a monoclonal antibody.
- 35 According to the invention, "low molecular weight substances" are naturally occurring or artificially produced molecules having a molecular weight of from about

250 to 1000 Da, preferably 300 to 750 Da, particularly preferably 400 to 600 Da, or are modified molecules of said molecular weight, which have been derived from natural substances.

5

The claimed invention furthermore comprises a primer having a sequence depicted in Table 1.

10 In addition, the invention relates to a primer pair having sequences depicted in Table 1, with the first primer of said primer pair serving as a forward primer and the second primer of said primer pair serving as a reverse primer to amplify a DNA sequence.

15 The primer of the invention (in combination with a further suitable primer, preferably a further suitable primer listed in Table 1) and the primer pair of the invention may be used for amplification of the sequences of the invention, preferably by means of PCR or LCR. The primers 20 and primer pairs, respectively, have been selected with great care from a multiplicity of potentially possible primers. Besides amplification of the nucleic acid sequences of the invention, they also allow amplification of sequences which encode enzymes of the prior art, and are 25 thus versatile.

The invention further relates to a kit comprising

the polypeptide of the invention;

30

the nucleic acid molecule of the invention;

the vector of the invention;

35 the host of the invention;

the ligand of the invention;

the reaction system of the invention;

at least one primer of the invention; and/or

5

at least one primer pair of the invention.

The components of the kit of the invention may be packaged individually or partly together in suitable vessels. The
10 components may be present in the kit of the invention, for example, in freeze-dried form or, for example, in solution, with suitable solvents including in particular aqueous solvents such as buffered solutions, for example phosphate-buffered solutions.

15

The kits of the invention may be used in many different ways. For example, they may serve to identify further alcohol dehydrogenases or nucleic acids encoding these, with preference being given to using the primers of the
20 invention. In other embodiments, the kits of the invention may be used for industrial production of the enzyme of the invention or of the products converted by said enzyme. In these embodiments, preference would be given to using the host of the invention or the reaction system of the
25 invention.

In the Figures:

Fig. 1: depicts the prior art via the resolution of the
30 racemate: at least 4-4 steps

Fig. 2: depicts an overview of cluster 2
(= primer group 2), based on 33 sequences

Fig. 3: depicts PCR typing with primer group 2,
using various pools

35

The examples illustrate the invention.

Example 1: Clustering of ADHs and primer design

5 Strains were prioritized from an extensive proprietary strain collection and grown in liquid culture after carrying out viability and purity checks. Harvested cell pellets served as starting material for genetic screening.
10 Primers for genetic screening for alcohol dehydrogenase genes were constructed and then tested on the basis of prepared genomic DNA of selected microbial isolates with the aid of PCR. NAD- or NADP-dependent alcohol dehydrogenases are classified as long-chain, medium-chain and short-chain ADHs. Since sequence heterogeneity within
15 these groups is substantial, said groups were grouped in clusters based on sequence analyses. The long-chain ADHs were divided into three clusters, the medium-chain ADHs in 4 clusters and the short-chain ADHs in three clusters. Subsequently, in each case four degenerated primer sets per
20 cluster were constructed which differ in the utilization of specific codons (codon usage) but which are directed against the same sequence motifs of the clusters.

Example 2:

25 Genetic screening for long-chain alcohol dehydrogenases

The long-chain ADHs were divided on the basis of sequence analyses into three clusters and the primers were
30 constructed and tested with an analogous procedure. However, despite expectations to the contrary, no PCR tags assignable to this group were amplified.

Example 3:

35 Genetic screening for medium-chain alcohol dehydrogenases

Primers directed against medium-chain ADHs were designed as follows: the medium-chain ADHs were divided based on sequence analysis into four clusters. Subsequently, in each case four degenerated primer sets were constructed which
5 differ by the selected codon usage. This will be illustrated graphically and by way of example in Figure 2 on the basis of the group of organisms for determining the primer group 2. The primer sets were selected on the basis of conserved regions in 33 different alcohol dehydrogenase
10 sequences.

These primer groups, for example primer group 2, were subsequently used for investigating various pools (containing genomic DNA from microorganisms). Using this
15 primer set, it was possible to amplify, clone and sequence novel partial medium-chain ADH sequences. The corresponding result of this PCR typing is depicted below in Figure 3. As documented, inter alia, by lanes 1, 2 and 10 in Figure 3, in each case here gene sequences were found which indicate
20 an alcohol dehydrogenase activity, owing to the gene sequence corresponding to known genes of ADH enzymes. Overall, further gene sequences with potential alcohol dehydrogenase activity were identified. The identity of the sequence tags found with already known ADHs was between 51
25 - 99%.

Example 4:

Genetic screening for special medium-chain alcohol
30 dehydrogenases, with analogy to alcohol dehydrogenases from *Rhodococcus* strains

Owing to the interesting properties of the known
Rhodococcus erythropolis (S)-alcohol dehydrogenase (S-Re-
35 ADH; this enzyme is characterized by stereoselective conversion of a broad spectrum of ketones and ketoesters to the corresponding hydroxy compounds) which likewise belongs

to the medium-chain alcohol dehydrogenases, and also of other alcohol dehydrogenases obtained from *Rhodococcus* strains, the question as to whether it is possible to identify novel ADH sequences exhibiting a relatively high 5 similarity to this sequence with the aid of genetic screening, was looked into. The driving force here is the assumption that novel ADHs whose sequences share a high identity with the S-Re-ADH sequence could likewise possess interesting properties. For example, such novel ADHs could 10 possess on the one hand the proven properties of S-Re-ADH but, on the other hand, for example, could have a modified substrate spectrum or increased expression performance. In order to answer the above question, comparative sequence analyses with the amino acid sequence of this S-Re-ADH were 15 carried out first. These analyses revealed that S-Re-ADH is a representative of cluster 1 of the medium-chain ADHs. Furthermore, a group consisting of 5 protein sequences, which includes S-Re-ADH, was found within this cluster. Starting from these 5 sequences, degenerated primers were 20 constructed and assayed, taking into account the codon usage, according to the procedure described above.

In order to reduce the number of PCRs to be carried out, pools consisting of 24 bacterial isolates were established 25 and DNA was isolated. This DNA was used as template. Numerous sequence tags were amplified and sequenced. Analysis of the sequence tags translated into amino acid sequences revealed identities to the S-Re-ADH sequence of from about 84% to 99%. Two full-length genes were isolated 30 which are represented by one sequence tag and which exhibit 98% identity to S-Re-ADH at the amino acid sequence level. The novel ADHs are derived from the organism *Arthrobacter paraffineus* ATCC21317. The homology at the DNA level is 94%. Said full-length genes were isolated with the aid of a 35 sequence homology approach.

Example 5:

Genetic screening for short-chain alcohol dehydrogenases

Moreover, 12 primer sets for the short-chain ADHs, which
5 are directed against the three clusters of this group, were
finally assayed. The template used was DNA which had been
isolated from 5 isolates which, owing to their ADH
activity, had reduced either 4-chloroacetophenone or 2-
heptanone in the activity screening. The identity of the
10 amino acid sequence tags to known short-chain ADH sequences
is between 47% and 84%, the vast majority of these
sequences exhibiting an identity of less than 67% to
published sequences.

Table 1: Primer sequences which were used for the screening of the DNA sequences encoding the alcohol dehydrogenases of the invention

	Name	Sequence 5' → 3'	Direction	Block
5	ADHM1:	AAAGCATGCGCGTTGYCAYACNGA	Forward	A
	ADHM2:	CCAATGTTCATCGCTTGATATGBNGTRATNCC	Reverse	C
	ADHM3:	TGCGGCGTCTGCCAYACBGA	Forward	A
	ADHM4:	GCTTCAGGGCGTGGTAGGBVGTVAYRCC	Reverse	C
10	ADHM5:	GCAGCGTCTGCCACWCSGA	Forward	A
	ADHM6:	GCTTCAGGGCCTGGTAGGBSGTSAYS SCC	Reverse	C
	ADHM7:	AGCCTGCGCGTCTGYCAYWC BGA	Forward	A
	ADHM8:	GCTTCAGCGCCTGGTAGGBSGTSAYNCC	Reverse	C
	ADHM9:	GCAGCTTGCAGGCAATGTGYCAYACNGA	Forward	A
15	ADHM10:	GCCCAAGCCGGTCGTAAYNCRCANCC	Reverse	C
	ADHM11:	GGCCTGCGGCATGTGYCAYACBGA	Forward	A
	ADHM12:	CCCAAGCCGGTCGTGAYRMMRCAVCC	Reverse	C
	ADHM13:	CCGGCATGTGCCACACSGA	Forward	A
	ADHM14:	TGGCGGCCAGGCCSAYSSCSCC	Reverse	C
20	ADHM15:	GGCCTCCGGCATGTGYCAYACSGA	Forward	A
	ADHM16:	TGGCGGCCAGGCCSAYNSCNCC	Reverse	C
	ADHM17:	TTAAATGGTGCAGGCATTGYGGNW CNGA	Forward	A
	ADHM18:	CAACTAACAGCCAACATGCCDATNGKNCC	Reverse	D

	<u>ADHM19 :</u>	<u>CAAGGTCAAGTGGTGCGBATYTGYGG</u>	Forward	A
	<u>ADHM20 :</u>	<u>TGACGGCCAACATGCCRATNGKVCC</u>	Reverse	D
	<u>ADHM21 :</u>	<u>TGCGGCATCTGCGGSWCSGA</u>	Forward	A
	<u>ADHM22 :</u>	<u>CGAACTTGACGGCGAAGAKSCCGATSGKSC</u>	Reverse	D
5	<u>ADHM23 :</u>	<u>CAAGGTCAAGTGGTGCNNATCTGYGG</u>	Forward	A
	<u>ADHM24 :</u>	<u>CGGCGAACATGCCGATSGKNCC</u>	Reverse	D
	<u>ADHM25 :</u>	<u>GATTGTTAGAGTTACAGCTACAGCTATTCYGGNWSNGA</u>	Forward	A
	<u>ADHM26 :</u>	<u>TGAACGGCAAACAGGCCNAYNGGNCC</u>	Reverse	D
	<u>ADHM27 :</u>	<u>CGCCACCGCCATCTGYGGBWSBGA</u>	Forward	A
10	<u>ADHM28 :</u>	<u>GACGGCGAACAGGCCNAYNGGVCC</u>	Reverse	D
	<u>ADHM29 :</u>	<u>CACCGCCATCTGCGGSWSSGA</u>	Forward	A
	<u>ADHM30 :</u>	<u>GGAGTGGACGGCGAACAKSCCSAYSGGSC</u>	Reverse	D
	<u>ADHM31 :</u>	<u>CGCCACCGCCATCTGYGGNWSBGA</u>	Forward	A
	<u>ADHM32 :</u>	<u>GACGGCGAACAGGCCSAYSGGNCC</u>	Reverse	D
15	<u>ADHM39</u>	<u>AGAAGAACTGGGCATTATGCCNCCNGGNYT</u>	Forward	A
	<u>ADHM40</u>	<u>TGTATCAATTGTCGGTTGATAGCCNACRAARTCNA</u>	Reverse	D
	<u>ADHM41</u>	<u>ACAACGTGGTCGTGTACGGNCCNTGGGG</u>	Forward	
	<u>ADHM42</u>	<u>GATGGTGGGCTGGTAGCCNACRAARTCNA</u>	Reverse	
	<u>ADHM43</u>	<u>GACAACGTGTCGTCTACGGNCCNTGGGG</u>	Forward	
20	<u>ADHM44</u>	<u>AGCGCTTGATGGCGTGRRTANGGNGT</u>	Reverse	
	<u>ADHM45</u>	<u>GACAACGTGTCGTCTACGGNCCNTGGGG</u>	Forward	

040057 AM / AL

- 36 -

ADHM46

GATGGTCGGCTGGTAGCCNACRAARTCNA

Reverse

ADHS1 : ADHS1 → ADHS24.

What is claimed is:

NO.: 18 or a sequence which is at least 70% identical to the sequence of SEQ ID NO.: 18, the sequence of SEQ ID NO.: 19 or a sequence which is at least 70% identical to the sequence of SEQ ID NO.: 19, the
5 sequence of SEQ ID NO.: 20 or a sequence which is at least 60% identical to the sequence of SEQ ID NO.: 20, the sequence of SEQ ID NO.: 21 or a sequence which is at least 90% identical to the sequence of SEQ ID NO.: 21, the sequence of SEQ ID NO.: 22 or a sequence which is at least 70% identical to the sequence of SEQ ID NO.: 22, the sequence of SEQ ID NO.: 23 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 23, the sequence of SEQ ID NO.: 24 or a sequence which is at least 65% identical to the
10 sequence of SEQ ID NO.: 24, the sequence of SEQ ID NO.: 25 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 25, the sequence of SEQ ID NO.: 26 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 26, the
15 sequence of SEQ ID NO.: 27 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 27, the sequence of SEQ ID NO.: 28 or a sequence which is at least 75% identical to the sequence of SEQ ID NO.: 28, the sequence of SEQ ID NO.: 29 or a sequence which is at least 70% identical to the sequence of SEQ ID
20 NO.: 29, the sequence of SEQ ID NO.: 30 or a sequence which is at least 60% identical to the sequence of SEQ ID NO.: 30, the sequence of SEQ ID NO.: 31 or a sequence which is at least 55% identical to the
25 sequence of SEQ ID NO.: 31, the sequence of SEQ ID NO.: 32 or a sequence which is at least 55% identical to the sequence of SEQ ID NO.: 32, the sequence of SEQ ID NO.: 33 or the sequence of SEQ ID NO.: 34.
30
35 2. A nucleic acid molecule, which encodes the polypeptide as claimed in claim 1.

3. A nucleic acid molecule, which is complementary to the nucleic acid molecule as claimed in claim 2.
4. A vector, which comprises the nucleic acid molecule as claimed in claim 2 or 3.
5
- 5 A nonhuman host, which comprises the polypeptide as claimed in claim 1 or the nucleic acid molecule as claimed in claim 2 or 3 or the vector as claimed in
10 claim 4.
6. The host as claimed in claim 5, which is a cell.
7. The host as claimed in claim 5, which is a transgenic
15 nonhuman animal.
8. The host as claimed in any of claims 5 to 7, which has a further dehydrogenase suitable for cofactor regeneration or a nucleic acid molecule encoding said
20 dehydrogenase.
9. The host as claimed in claim 8, in which the dehydrogenase suitable for cofactor regeneration is a formate dehydrogenase or a glucose dehydrogenase.
25
10. A reaction system, comprising an organic compound which is a substrate of a dehydrogenase, the polypeptide as claimed in claim 1, the vector as claimed in claim 4 or the host as claimed in any of claims 5 to 9 and, where appropriate, a cofactor for
30 the polypeptide as claimed in claim 1.
11. The reaction system as claimed in claim 10, in which the organic compound which is a substrate of a dehydrogenase is a carbonyl compound.
35

12. The reaction system as claimed in claim 11, in which the carbonyl compound is an aldehyde or a ketone.
13. The reaction system as claimed in claim 12, in which
5 the ketone is an asymmetrically substituted ketone.
14. The reaction system as claimed in claim 10, in which the organic compound which is a substrate of a dehydrogenase is an alcohol.
- 10
15. The reaction system as claimed in claim 14, in which the alcohol is a primary alcohol or a chiral secondary alcohol.
- 15 16. The reaction system as claimed in any of claims 10 to 15, in which the cofactor is NADH, NADPH, NAD⁺ or NADP⁺.
17. A process for preparing the polypeptide as claimed in
20 claim 1 or of a polypeptide encoded by the nucleic acid molecule as claimed in claim 2, which process comprises growing the host as claimed in any of claims 5 to 9 and isolating said polypeptide.
- 25 18. A process for preparing the polypeptide as claimed in claim 1 or of a polypeptide encoded by the nucleic acid molecule as claimed in claim 2, which process comprises isolating said polypeptide from a body fluid or tissue sample of the host as claimed in any of
30 claims 7 to 9.
- 19 A process for an organic compound which is a product of a dehydrogenase, which process comprises reacting an organic compound which is a substrate of a
35 dehydrogenase with the polypeptide as claimed in claim 1, the host as claimed in any of claims 5 to 9 or by

means of the reaction system as claimed in any of claims 10 to 16.

20. The process as claimed in claim 19, which further comprises the step of isolating the product of the reaction.
- 5 21. The process as claimed in claim 20, which further comprises processing the product to give a medicament.
- 10 22. The process as claimed in claim 20, further comprising the step of processing the product to give a secondary product.
- 15 23. The process as claimed in claim 22, further comprising the step of formulating the secondary product in the preparation of a medicament.
- 20 24. The process as claimed in any of claims 19 to 23, in which the product is an enantiomerically pure alcohol.
- 25 25. A ligand, which specifically binds the polypeptide as claimed in claim 1, which ligand is neither a substrate or cofactor of said polypeptide nor a product generated therefrom.
- 30 26. The ligand as claimed in claim 25, which is an antibody or a fragment or derivative thereof, an aptamer, or a low-molecular weight substance.
27. A primer, having a sequence depicted in Table 1.
- 35 28. A primer pair, having sequences depicted in Table 1, with the first primer of said primer pair serving as a forward primer and the second primer of said primer pair serving as a reverse primer to amplify a DNA sequence.

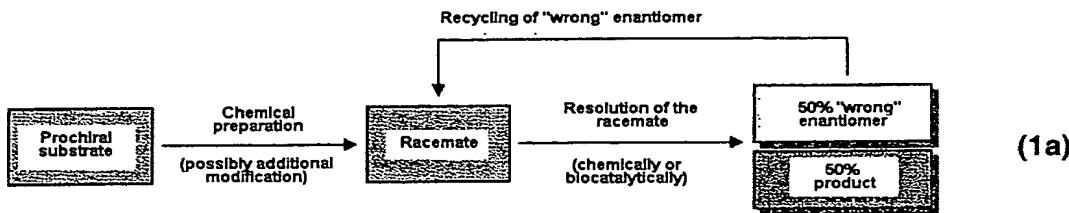
29. A kit, comprising

- (a) the polypeptide as claimed in claim 1;
- 5 (b) the nucleic acid molecule as claimed in claim 2 or 3;
- (c) the vector as claimed in claim 4;
- 10 (d) the host as claimed in any of claims 6 to 9;
- (e) the reaction system as claimed in any of claims 10 to 16;
- 15 (f) the ligand as claimed in claim 25 or 26;
- (g) at least one primer as claimed in claim 27; and/or
- 20 (h) at least one primer pair as claimed in claim 28.

Abstract

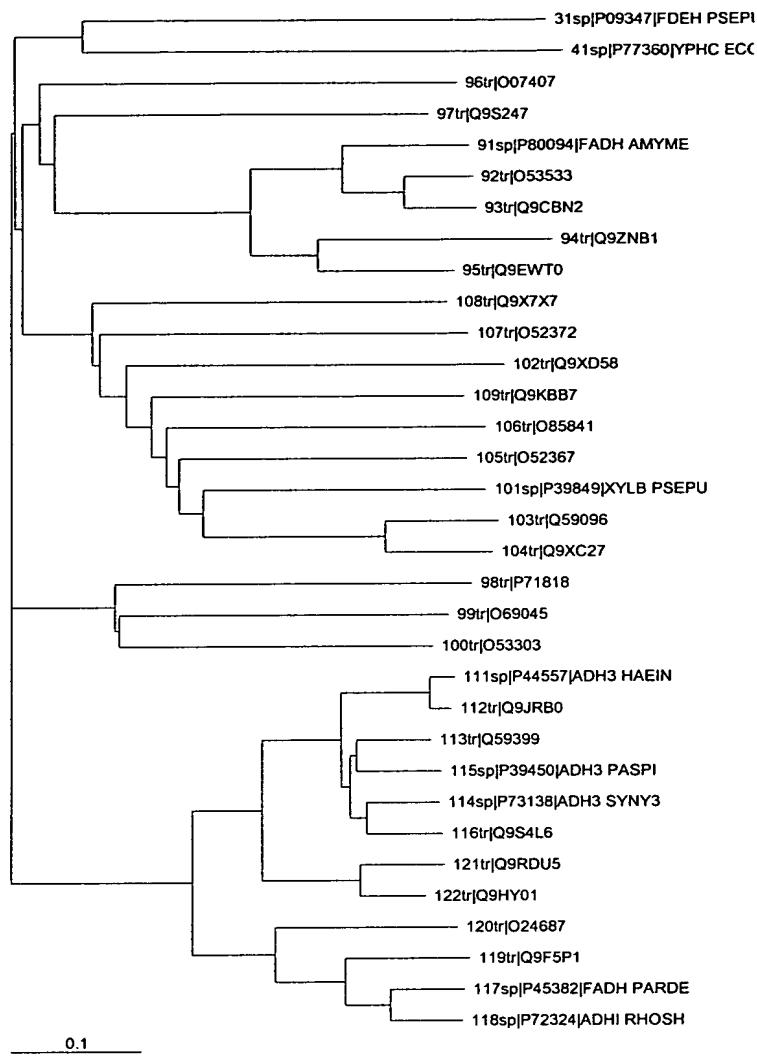
The invention relates to novel polypeptides which have the biological activity of an NAD- or NADP-dependent alcohol dehydrogenase. The invention furthermore relates to nucleic acids encoding said polypeptides, to nonhuman hosts or host cells and to reaction systems which may be used for preparing desired products. The polypeptides of the invention are preferably used in the preparation, starting from aldehydes or ketones, of primary and enantiomerically pure secondary alcohols which may serve as intermediates for medicaments. Alternatively, the polypeptides of the invention may also be employed in the reverse reaction, i.e. the oxidation of alcohols with the formation of aldehydes or ketones.

- Prior art via resolution of the racemate: at least 3-4 steps

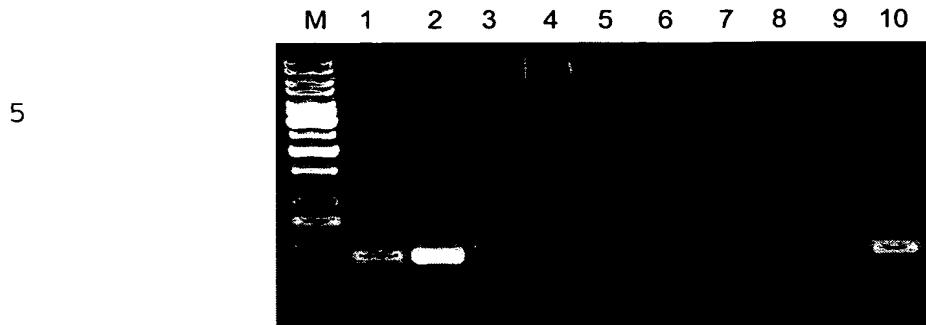


- Biocatalytic and sustainable concept: Asymmetrical biocatalysis



**Fig. 2.**

Overview of cluster 2 (= primer group 2), based on 33 sequences



10 Fig. 3

PCR typing with primer group 2, using various pools

Lane occupation: M: Marker 1 kb DNA ladder; Lane 1: Pool 1
with primers ADHM9 + 10; Lane 2: Pool 1 with primers ADHM11
+ 12; Lane 3: Pool 1 with primers ADHM13 + 14; Lane 4: Pool
15 1 with primers ADHM15 + 16; Lane 5: Pool 2 with primers
ADHM 9 + 10; Lane 6: Pool 2 with primers ADHM11 + 12; Lane
7: Pool 2 with primers ADHM13 + 14; Lane 8: Pool 2 with
primers ADHM15 + 16; Lane 9: Pool 3 with primers ADHM9 +
10; Lane 10: Pool 3 with primers ADHM11 + 12